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Advancing environmental monitoring: Rapid quantitation of 28 PFAS in aquatic insect tissue using QuEChERS extraction coupled with UPLC-MS/MS

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ABSTRACT

Per- and polyfluoroalkyl substances (PFAS) have raised global concern due to their high environmental persistence, potential to bioaccumulate, and toxicity. Recent studies have focused on PFAS in aquatic larval insects due their sustained contact with potentially contaminated water and high importance as food subsidies to higher order consumers. Although previous studies have validated rapid methods for PFAS detection in various media, only a few have focused on method development and quantitation in insect tissue. The present study employed a Quick, Easy, Cheap, Effective, Rugged, and Safe (QuEChERS) sample extraction approach, followed by ultra-performance liquid chromatography tandem mass spectrometry (UPLC-MS/MS) for the identification and quantitation of 28 PFAS in nymphal dragonfly tissues. This method was validated using method detection limits and precision and accuracy studies. Recoveries ranged from 71.0% to 102.8%, whereas relative standard deviations spanned 2.0% to 4.6% at the 20.0 ng mL^{-1} analyte level, and method detection limits ranged from 0.5 ng g^{-1} to 2.0 ng g^{-1}. Analysis of 15 wild-caught nymphal dragonfly samples resulted in the detection of perfluorooctanesulfonic acid (PFOS), perfluorooctanoic acid (PFOA), perfluorononanoic acid (PFNA), perfluoroundecanoic acid (PFUnA), perfluorohexanesulfonic acid (PFHxS), perfluorodecanoic acid (PFDA), perfluorohexanoic acid (PFHxA), and 1H,1H,2H,2H-perfluorooctanesulfonic acid (6:2FTS), with PFOS detected in every sample. This validated method displayed strong recovery across multiple functional groups and provided an efficient and effective method for PFAS detection in aquatic larval insects.

KEYWORDS: dragonfly nymph, odonata, per- and polyfluoroalkyl substances (PFAS), QuEChERS, UPLC-MS/MS, aquatic insects.

INTRODUCTION

Emerging contaminants, such as per- and polyfluoroalkyl substances (PFAS), have gained increasing attention in recent years due to their widespread detection in environmental media and their association with negative health impacts. PFAS have amphiphilic and oleophobic properties which underlie their use in numerous applications and consumer products [1], including chrome plating, pesticides [2], stain and water-resistant fabrics [3], aqueous film-forming foam [4], and personal protective equipment [5]. The strong carbon-fluorine bond in PFAS renders them resistant to biological, chemical, and environmental degradation, resulting in persistence in the environment and lending the nickname “forever chemicals”. These unique properties contribute to

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PFAS bioaccumulation and biomagnification in food webs [6, 7], warranting concern for upper trophic level consumers including humans.

Due to the high-water solubility of many PFAS, recent research has largely focused on detection in aquatic media and biota. Aquatic insects are of particular interest due to their relatively sedentary lifestyle, sustained contact with sediments, and ability to bioaccumulate a variety of contaminants [8, 9]. Moreover, aquatic insects serve as important prey and sources of contaminant exposure for aquatic and terrestrial predators such as fish, amphibians, riparian spiders, birds, and bats. [10-15]. Several studies have demonstrated PFAS bioaccumulation in aquatic larval insects from the Hudson River Watershed (Hudson, NY, USA) [16], Yadkin-Pee Dee River (North and South Carolina, USA) [17], Windermere Basin (Hamilton, Ontario, Canada) [13], South Africa [18], and Sweden [19], indicating widespread contamination and interest in PFAS analysis of freshwater aquatic insects.

Among aquatic insects, larval dragonflies (i.e., nymphs) have been used as biosentinels for metal contamination, including mercury contamination, due to their widespread geographical range, long lifespan (2-5 years), high site fidelity, and bioaccumulation potential [20-22]. PFAS are known to bind to proteins and biopolymers such as chitin, which makes up the mouthparts and exoskeleton of dragonfly nymphs [23]. Additionally, dragonfly nymphs are voracious predators, and their diet may consist of other aquatic insects and appropriately sized fish and tadpoles [24]. Dietary pathways are important exposure routes for contaminants including PFAS [25]. PFOA and PFOS concentrations up to 1.3 ng g^{-1} and 8.4 ng g^{-1} have been detected in dragonfly nymphs in Tanzania, respectively, whereas PFOS concentrations up to 4.4 ± 3.1 ng g^{-1} have been detected in nymphs from the United States [16, 26]. Previous PFAS detections in dragonfly nymphs along with inexpensive collection methods, easy taxonomic identification, and lack of any permit requirement for their collection, further increases their potential as bioindicators of PFAS contamination in aquatic ecosystems.

As aquatic insects, such as dragonfly nymphs, are increasingly used as bioindicators of environmental contamination, it becomes vital to develop and validate simplistic, efficient, and reliable detection methods. Numerous studies have employed generic extraction methods for the detection and quantitation of PFAS in insect tissue; however, these studies fail to provide tissue-specific recovery levels, detection limits, or matrix matching, which may lead to biased or unreliable measurements [26, 27]. Importantly, there was a single study that used proper matrix matching during validation, highlighting the value of such approach, although they only analyzed PFOS and PFOA [28]. The quick, easy, cheap, effective, rugged, and safe (QuEChERS) extraction method [29] has been previously used for the analysis of veterinary drugs, mycotoxins, and pesticides in various insect species and is often touted as an accurate extraction method at ultra-trace levels [30-32]. Although QuEChERS has been previously used for the analysis of pesticides in dragonfly nymphs [33], we are unaware of any studies that have validated a QuEChERS-based approach for PFAS analysis in nymphs.

The purpose of the present study is to develop and validate a rapid and sensitive analytical method for the analysis of 28 PFAS in dragonfly nymphs using QuEChERS extraction followed by UPLC-MS/MS analysis. This method allows the simultaneous analysis of a diverse array of analytes, enabling reliable and rapid screening of batched samples. This method also benefits from low sample masses (0.5 g), which may allow for the analysis of individual nymphs depending on instar stage. Overall, the proposed method demonstrated good recovery and could be used to monitor PFAS in freshwater ecosystems using dragonfly nymphs.

MATERIALS AND METHODS

Methanol (≥ 99.9%; HPLC grade), ammonium acetate (≥ 97%; HPLC grade), Optima water (LC/MS grade; W64), and ACS grade acetone were purchased from Ricca Chemical Company (Arlington, TX, USA). The internal standard (EPA-537IS) and the surrogate (EPA-537SS-R1) solution were purchased from Wellington Laboratories (Guelph, Ontario, Canada). The following standards were purchased in the form of EPA 537.1 Method Standard from AccuStandard,

Inc. (New Haven, CT, USA): hexafluoropropylene oxide dimer acid (HFPO-DA, GenX), N-ethylperfluorooctane sulfonamidoacetic acid (NEtFOSAA), N-methylperfluorooctane sulfonamidoacetic acid (NMeFOSAA), perfluorobutanesulfonic acid (PFBS), perfluorodecanoic acid (PFDA), perfluorododecanoic acid (PFDoA), perfluoroheptanoic acid (PFHpA), perfluorohexanesulfonic acid (PFHxS), perfluorohexanoic acid (PFHxA), perfluorononanoic acid (PFNA), perfluorooctanesulfonic acid (PFOS), perfluorooctanoic acid (PFOA), perfluorotetradecanoic acid (PFTA), perfluorotridecanoic acid (PFTrDA), perfluoroundecanoic acid (PFUnA), 11-chloroeicosafluoro-3-oxaundecane-1-sulfonic acid (11Cl-PF3OUdS), 9-chlorohexadecafluoro-3-oxanonane-1-sulfonic acid (9Cl-PF3ONS), and 4,8-dioxa-3H-perfluorononanoic acid (ADONA). The Perfluorinated Native Compound Standard (PFC-24-10X) was also purchased from AccuStandard, Inc. and contained the following standards: perfluorohexanoic acid (PFHxA), perfluoroheptanoic acid (PFHpA), perfluorooctanoic acid (PFOA), perfluorononanoic acid (PFNA), perfluorodecanoic acid (PFDA), perfluoroundecanoic acid (PFUnA), perfluorododecanoic acid (PFDoA), perfluorotridecanoic acid (PFTrDA), perfluorotetradecanoic acid (PFTA), N-methyl perfluorooctanesulfonamidoacetic acid (NMeFOSAA), N-ethyl perfluorooctanesulfonamidoacetic acid (NEtFOSAA), perfluorobutanoic acid (PFBA), perfluoropentanoic acid (PFPeA), perfluorobutanesulfonic acid (PFBS), perfluoropentane sulfonic acid (PFPeS), perfluorohexanesulfonic acid (PFHxS), perfluoroheptanesulfonic acid (PFHpS), perfluorononanesulfonic acid (PFNS), perfluorooctanesulfonic acid (PFOS), perfluorodecanesulfonic acid (PFDS), 1H,1H,2H,2H-perfluorohexanesulfonic acid (4:2FTS), 1H,1H,2H,2H-perfluorooctanesulfonic acid (6:2FTS), 1H,1H,2H,2H-perfluorodecanesulfonic acid (8:2FTS), and perfluorooctane sulfonamide (PFOSA). The PFAS 28 Calibration Standard was purchased from Restek (Bellefonte, PA, USA; product # 30734) and was used as a second source. Polypropylene (PP) Corning™ centrifuge tubes with high density polyethylene (HDPE) CentriStar™ caps, and polyethylene (PE) Frutti Treasure® gloves were purchased from Fisher Scientific (Waltham, MA, USA). LC vials with PP caps were obtained from Waters (Milford, MA, USA). PP centrifuge tubes (2.0 mL) were purchased from Eppendorf (Hauppauge, NY, USA). QuEChERS in the form of $MgSO_4$/NaCl (ECMSSC-MP) and PSA/GCB/$MgSO_4$ (ECMSCB-MP) were purchased from UTC (Bristol, PA, USA).

Instrumentation

Initial weighing was conducted using a Sartorius Cubis® II Semi Micro Balance (MCA225S-2S00-I; Göttingen, Germany) with a sensitivity of 0.00001 g. Subsequently, samples were subjected to sonication employing a Branson 5510R-DTH ultrasonic cleaner (Danbury, CT, USA), followed by vortexing using a Fisher Scientific 02215452 multitube vortexer (Waltham, MA, USA) to ensure thorough homogenization. Further processing involved centrifugation, performed with precision using both the Thermo Scientific CL10 centrifuge (model # 11210901) and the Thermo Scientific Legend Micro 21R centrifuge (model # 75002446; Waltham, MA, USA). The analysis was conducted on a Waters Acquity UPLC-MS/MS system coupled with a triple-quadrupole (TQD) tandem mass spectrometer (Waters Corp., Milford, MA, USA) for the comprehensive examination of 28 PFAS compounds of interest. To safeguard against potential sample contamination, the analytical instrument was retrofitted with a PFAS kit from Waters Corp. (SKU #176004554 Milford, MA, USA).

Preparation of standard solutions

All solutions were prepared within a controlled PFAS-free cleanroom environment while utilizing polyethylene (PE) gloves to prevent potential cross-contamination from nitrile gloves. Furthermore, to ensure the utmost purity of glassware, each vessel underwent a rigorous triple rinse with deionized water, followed by an additional rinse with ACS grade acetone, and was subsequently baked at 500 °C overnight to eliminate any residual contaminants. Two calibration solutions

were prepared by serially diluting both the EPA 537.1 and PFC-24-10X Method Standards using a 96:4% (vol/vol) methanol:water mixture (Table S1 and S2). The surrogate stock solution, consisting of concentrations of 1000 ng mL^{-1} MPFHxA, 1000 ng mL^{-1} MPFDA, 1000 ng mL^{-1} M3HFPO-DA, and 4000 ng mL-1 d5-N-EtFOSAA, was procured commercially and utilized for surrogate calibration (Table S3 and S4). Each calibration curve was generated to ensure accuracy, employing pure standard calibration curves rather than matrix-matched curves. The EPA-537SS-R1 stock solution served as the surrogate spiking solution, whereas EPA-537IS was used as the internal standard solution and was added to each sample to facilitate precise quantitation.

Sample preparation and clean-up

Live dragonfly nymphs were purchased from Carolina Biological Supply (*Libellulida* species, product #143526) and shipped overnight to the University of Connecticut (Storrs, CT, USA). Immediately upon arrival, individuals were rinsed with Optima water, stored in 50 mL HDPE falcon tubes, and frozen at -20 °C until further processing. For sample preparation, nymphs were thawed at room temperature and one to two individuals were homogenized per sample with a methanol and Optima water-rinsed stainless steel spatula. An aliquot of 0.5 ± 0.0001 g (ww; wet weight) was weighed and transferred into a new 2.0 mL PP centrifuge tube.

Sample extraction and clean-up procedures closely followed those outlined by Campbell *et al.* [34]. Nymph samples were spiked with 20 µL of surrogate solution (EPA-537SS-R1), for a final concentration of 20 ng mL^{-1} for MPFHxA, MPFDA, and M3HFPO-DA, and 80 ng mL^{-1} for d5-N-EtFOSAA. For quality control (QC) purposes, previously analyzed PFAS-free dragonfly nymph tissue samples were used as method blanks, matrix spikes (MS), and matrix spike duplicates (MS-DUP) to assess precision and bias. A final concentration of 100 ng mL^{-1} was achieved for MS, MS-DUP, and laboratory control samples (LCS) by fortification with 100 µL of the 1000 ng mL^{-1} standard solution. During the early stages of the present study a single standard solution containing all 28 PFAS analytes of interest was unavailable; therefore the chosen standards (EPA 537.1 Method Standard and PFC-24-10X) contained overlapping analytes.

Samples underwent a series of preparation steps as follows: initially, vortexing for one minute at 2500 rounds per minute (rpm) was carried out with pulse mode off. Subsequently, 980 µL of methanol was added to each sample and 880 µL to the QC samples. Following an additional vortexing cycle lasting one minute at 2500 rpm and a one-minute sonication step, each sample received 0.1-0.3 g of QuEChERS (MgSO4/NaCl), followed by physical hand shaking and a subsequent vortexing session lasting 10 minutes at 2500 rpm. The samples were then cooled overnight at -20 °C to facilitate sample clean-up by encouraging the precipitation of unwanted matrix interferences. The following day, centrifugation was conducted at 14,000 rpm for 10 minutes and a 500 µL aliquot of the supernatant was transferred to a 2.0 mL PP centrifuge tube. Then 0.1-0.3 g of QuEChERS clean-up powder (PSA/GCB/MgSO4) was added and samples were vortexed for 10 minutes at 2500 rpm, centrifuged for 10 minutes at 14,000 rpm, and 190 µL of the supernatant was transferred to a 300 µL LC vial. Finally, the samples were spiked with 10 µL of internal standard (EPA-537 IS) and vortexed to achieve final concentrations of 40 ng mL^{-1} for M2PFOA, 120 ng mL^{-1} for MPFOS, and 160 ng mL^{-1} for d3-N-MeFOSAA.

Sample analysis

Sample analysis was conducted using a Waters Acquity UPLC-MS/MS system, specifically configured for the detection of 28 PFAS compounds (refer to Table S5). The system was equipped with a Waters Acquity UPLC BEH C18 column (1.7 µm, 2.1 x 100 mm) that was preconditioned with 100% methanol for 60 minutes prior to sample analysis. The column was maintained at a constant temperature of 40 °C throughout the analysis, with an injection volume of 5 µL. The mobile phase consisted of a mixture of 95:5 H_2O/MeOH with 20 mM ammonium acetate (solvent A) and 100% MeOH (solvent B). The chromatographic separation was achieved using a gradient elution method over a total run

time of 12 minutes at a constant flow rate of 0.3 mL min^{-1}. Initially, the gradient started at 50% B, held for 0.3 minutes, then increased linearly to 99% B until 9 minutes. Subsequently, the column was reconditioned to the initial state within 10 seconds and maintained at 50% B until the end of the run at 12 minutes. The electrospray ionization (ESI) source was operated in negative ionization mode. Multiple reaction monitoring (MRM) transitions and retention times were used for analyte identification. These parameters along with cone voltage and collision energy for the 28 target PFAS compounds are detailed in Table 1. MRM chromatograms for analytes of interest can be found in Figure 1, whereas internal standards and surrogate solutions are illustrated in Figure 2. Instrumental parameters for the mass spectrometer were optimized as follows: capillary voltage 3.6 kV, cone voltage 40 V, desolvation temperature 350 °C, source temperature 150 °C, desolvation gas flow 700 L/hr, and collision gas flow 0.2 mL min^{-1}. Data acquisition and processing were conducted using Waters MassLynx software (Version 4.1), enabling statistical analysis and comprehensive data interpretation.

RESULTS AND DISCUSSION

This study aimed to establish and validate a robust method for the detection and quantitation of 28 PFAS compounds in dragonfly nymphs, while also minimizing steps, reagent volumes, and processing time. Chromatographic separation of the 28 PFAS was achieved efficiently within 12 minutes using a Waters Acquity UPLC BEH C_{18} (1.7 µm, 2.1 x 100 mm) column, eliminating the need for evaluating alternative columns. A nine-point calibration curve spanning a concentration range of 5.0 to 1,000 ng mL^{-1} for each PFAS was generated, exhibiting excellent linearity with correlation coefficients (R^2) of 0.9974 or higher (Table 2). Four dragonfly nymph replicates at the 20 ng mL^{-1} concentration level were used in the PA intra-day study (Table 3), whereas seven nymph replicates at the 5 ng mL^{-1} concentration level were used in the MDL intra-day study (Table 4). The method detection limit was calculated using the formula: MDL = (student's t value for seven replicates at the 99% confidence level) x (the standard deviation (SD) of the replicate analysis); where student's t value was determined to be 3.143. Various quality parameters

Table 1. Multiple-reaction monitoring (MRM) parameters, cone voltage (V), collision energies (eV), and retention times (RT; min) for the identification and quantification of 28 per- and polyfluoroalkyl substances (PFAS) in dragonfly nymph tissue.

Analyte	MRM transition (m/z)	Cone voltage (V)	Collision energy (eV)	RT (min)
PFBA	213.0 > 169.0	22.0	10.0	1.24
PFPeA	263.0 > 219.0	20.0	8.0	1.85
PFBS	299.0 > 80.0	42.0	30.0	1.94
PFHxA	313.0 > 269.0	14.0	10.0	2.76
$13C_2$-PFHxA (sur)	315.0 > 270.0	16.0	10.0	-
4:2 FTS	327.0 > 307.0	40.0	18.0	2.69
PFPeS	349.0 > 80.0	45.0	35.0	2.87
$13C_2$-HFPO-DA GenX (sur)	287.0 > 169.0	10.0	6.0	-
HFPO-DA GenX	285.0 > 169.0	12.0	8.0	2.99
ADONA	377.0 > 251.0	14.0	12.0	3.74
PFHpA	363.0 > 319.0	12.0	10.0	3.68
PFHxS	399.0 > 80.0	46.0	32.0	3.52, 3.74

Table 1 continued..

Analyte	MRM transition (m/z)	Cone voltage (V)	Collision energy (eV)	RT (min)
$13C_2$-PFOA (IS)	415.0 > 370.0	16.0	10.0	-
PFOA	413.0 > 369.0	14.0	10.0	4.49
PFHpS	449.0 > 80.0	45.0	40.0	4.54
6:2 FTS	427.0 > 407.0	47.0	22.0	4.46
9Cl-PF3ONS	531.0 > 351.0	34.0	24.0	5.49
PFNA	463.0 > 419.0	16.0	12.0	5.18
PFOS	499.0 > 80.0	52.0	42.0	4.98, 5.19
$13C_4$-PFOS (IS)	503.0 > 80.0	58.0	42.0	-
PFNS	549.0 > 80.0	45.0	30.0	5.78
8:2 FTS	527.0 > 507.0	53.0	28.0	5.76
PFDA	513.0 > 469.0	14.0	10.0	5.76
$13C_2$-PFDA (sur)	515.0 > 470.0	18.0	10.0	-
d_3-NMeFOSAA (IS)	573.0 > 419.0	28.0	14.0	-
NMeFOSAA	570.0 > 419.0	30.0	20.0	6.03
PFOSA	498.0 > 78.0	45.0	40.0	6.19
PFDS	599.0 > 80.0	45.0	30.0	6.26
d_5-NEtFOSAA (sur)	589.0 > 419.0	28.0	22.0	-
NEtFOSAA	584.0 > 419.0	30.0	20.0	6.07, 6.26
11Cl-PF3OUdS	631.0 > 451.0	40.0	24.0	6.46
PFUnA	563.0 > 519.0	12.0	10.0	6.26
PFDoA	613.0 > 569.0	18.0	10.0	6.68
PFTrDA	663.0 > 619.0	14.0	14.0	7.04
PFTA	713.0 > 669.0	14.0	12.0	7.35

sur = surrogate; IS = internal standard.

were assessed, including accuracy (recovery %), precision, and measurement uncertainty (MU). Sample concentrations were reported without correction for surrogate recovery. Accuracy was evaluated using the spike recovery method, where the mean calculated concentration of the analyte was compared to the nominal concentration of the spike. Precision was determined by calculating the relative standard deviation (RSD). Measurement uncertainty (MU) was computed dividing the RSD (%) from the PA study by 100 and multiplying by 2.

PFAS MDLs ranged from 0.5 ng g^{-1} to 2.0 ng g^{-1}, with accuracy levels varying from 71.0-102.8% (Tables 3 and 4). All analytes demonstrated recoveries within the acceptable range of 60-125% (Table 3) [35]. PFPeA had the lowest recovery at 70.1%, whereas PFTA (100.5%) and PFHpS (102.8%) were the only analytes that had recoveries ≥100% (Table 3). RSD ranged from 2.0% to 4.6% at the 20.0 ng mL^{-1} spiking concentration level and most analytes had RSDs <4.0%. Koch et al. [36] reported lower MDLs for

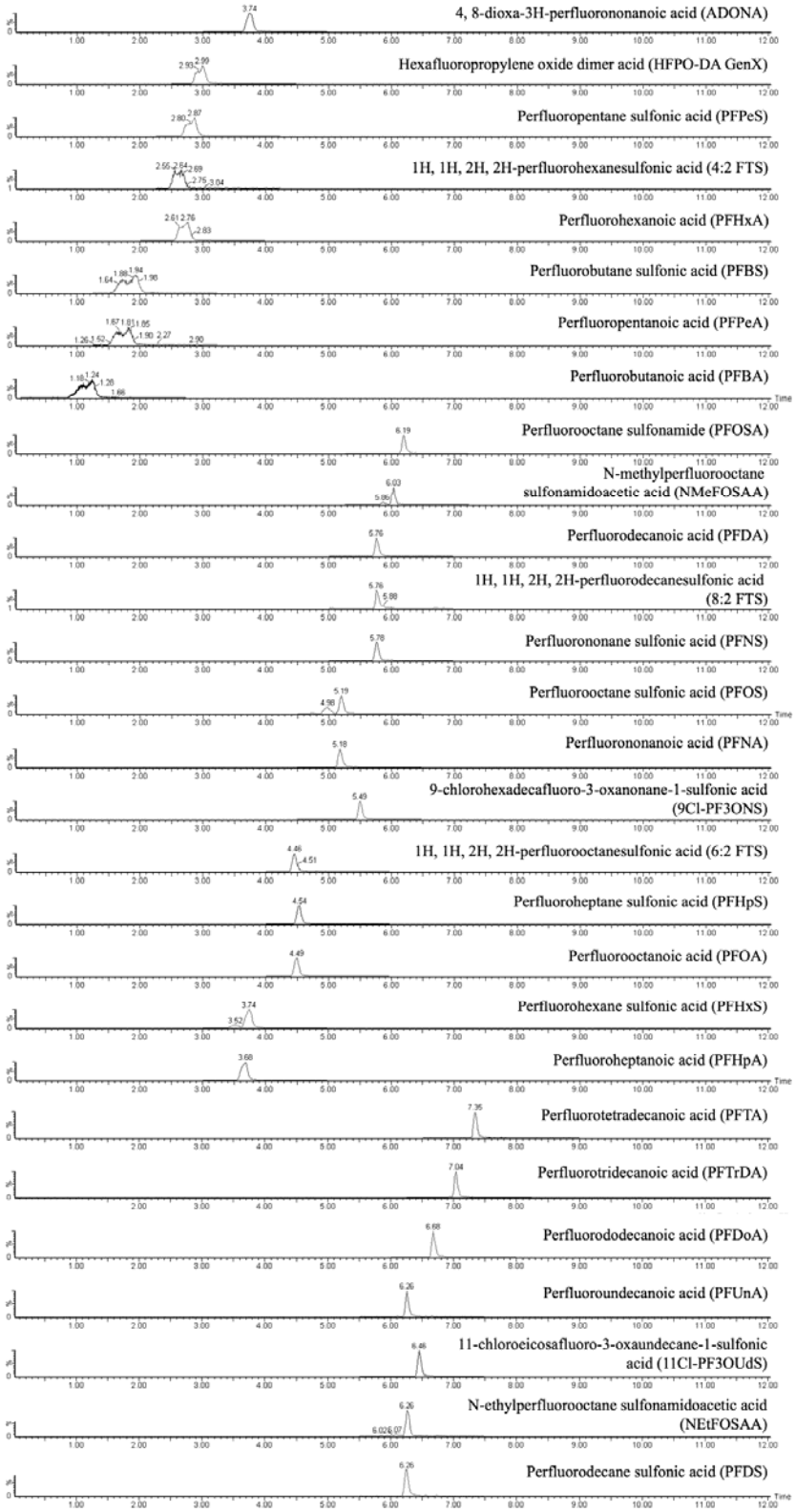

Figure 1. Chromatograms showing multiple reaction monitoring (MRM) for the detection and separation of 28 per- and polyfluoroalkyl substances (PFAS).

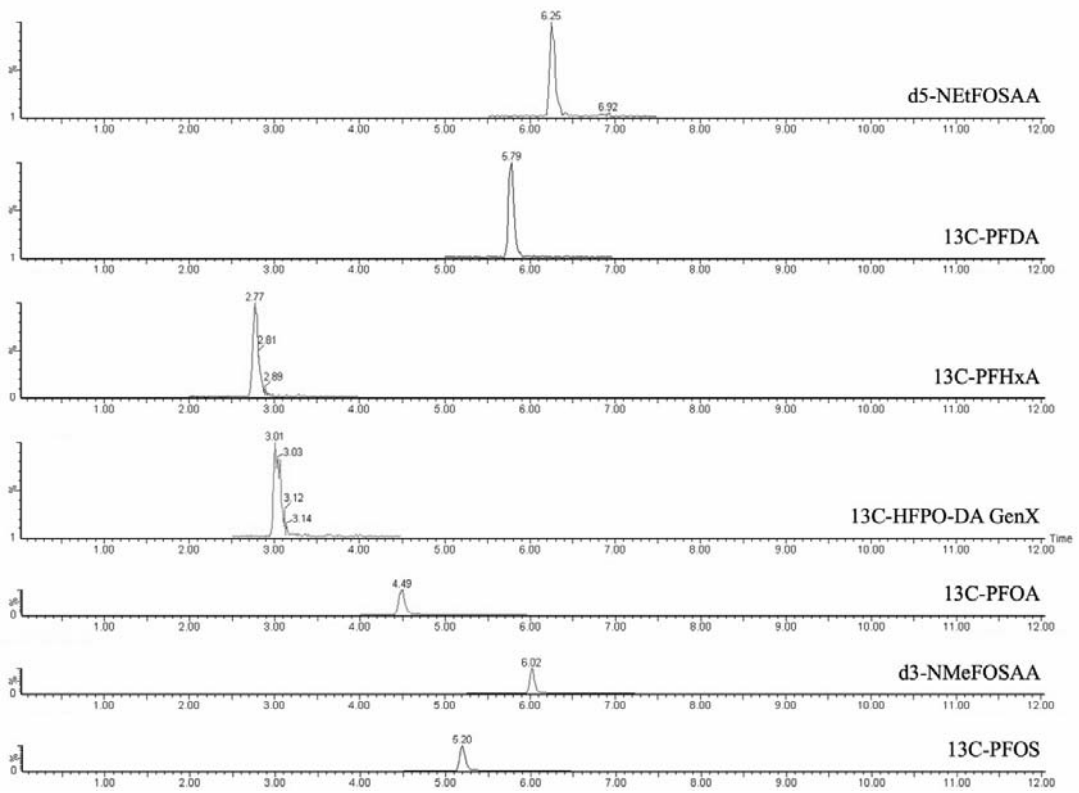

Figure 2. Chromatograms showing multiple reaction monitoring (MRM) for the detection and separation of surrogates (EPA-537SS-R1) and internal standards (EPA-537IS) utilized in this study.

Table 2. Correlation coefficients (R^2) for 28 per- and polyfluoroalkyl substances (PFAS) in dragonfly nymph tissue. Analytes are defined in Table S5 [34].

PFAS*	**Correlation coefficient (R^2)**
PFBA	0.9987
PFPeA	0.9998
PFBS	0.9984
PFHxA	0.9998
4:2FTS	0.9993
PFPeS	0.9996
HFPO-DA GenX	0.9996
ADONA	0.9996
PFHpA	0.9992
PFHxS	0.9999
PFOA	0.9997

Table 2 continued..

PFAS*	Correlation coefficient (R^2)
PFHpS	0.9999
6:2FTS	0.9995
9Cl-PF3ONS	0.9998
PFNA	0.9998
PFOS	0.9993
PFNS	0.9998
8:2FTS	0.9998
PFDA	0.9998
NMeFOSAA	0.9981
PFOSA	0.9974
PFDS	0.9997
NEtFOSAA	0.9993
11Cl-PF3OUdS	0.9999
PFUnA	0.9995
PFDoA	0.9999
PFTrDA	0.9996
PFTA	0.9998

Table 3. Precision (relative standard deviation, RSD, intra-day study) and accuracy (recovery %) study with measurement uncertainty (MU) for 28 per- and polyfluoroalkyl substances (PFAS) in dragonfly nymph tissue (n = 4). Analytes are defined in Table S5 [34].

PFAS*	Spike concentration (ng mL^{-1})	Recovery, %	RSD (%)	MU
PFBA	20	76.8	2.3	4.6
PFPeA	20	71.0	2.6	5.2
PFBS	20	78.9	3.6	7.2
PFHxA	20	79.5	3.0	6.0
4:2FTS	20	93.9	4.6	9.1
PFPeS	20	99.0	2.4	4.8
HFPO-DA GenX	20	91.3	2.6	5.3
ADONA	20	97.4	4.0	8.0
PFHpA	20	86.4	2.5	5.0
PFHxS	20	94.5	2.0	4.0

Table 3 continued..

PFAS*	Spike concentration (ng mL^{-1})	Recovery, %	RSD (%)	MU
PFOA	20	91.0	2.8	5.7
PFHpS	20	102.8	2.8	5.6
6:2FTS	20	92.4	2.5	4.9
9Cl-PF3ONS	20	72.3	3.1	6.2
PFNA	20	76.3	4.4	8.7
PFOS	20	96.8	3.8	7.6
PFNS	20	97.9	2.5	5.0
8:2FTS	20	86.1	3.1	6.3
PFDA	20	98.3	3.0	6.0
NMeFOSAA	20	74.8	3.2	6.4
PFOSA	20	79.6	2.2	4.4
PFDS	20	93.5	3.5	7.1
NEtFOSAA	20	73.9	3.6	7.3
11Cl-PF3OUdS	20	73.3	4.6	9.1
PFUnA	20	96.4	2.0	3.9
PFDoA	20	98.8	3.5	7.0
PFTrDA	20	97.0	3.5	7.0
PFTA	20	100.5	2.7	5.4

*Mass used for all samples is 0.5 g.

Table 4. Method detection limits (MDLs) and recovery ± relative standard deviation (RSD) for 28 per- and polyfluoroalkyl substances (PFAS) in dragonfly nymph tissue (n= 7). The analytes are defined in Table S5 [34].

PFAS*	Spike concentration (ng mL^{-1})	Recovery ± RSD	MDL (ng g^{-1})
PFBA	5.0	97.1 ± 5.5	1.7
PFPeA	5.0	101.4 ± 2.0	0.6
PFBS	5.0	95.4 ± 3.5	1.0
PFHxA	5.0	98.0 ± 6.0	1.8
4:2FTS	5.0	106.6 ± 4.0	1.3
PFPeS	5.0	89.1 ± 3.4	0.9
HFPO-DA GenX	5.0	94.6 ± 1.9	0.6
ADONA	5.0	104.9 ± 3.7	1.2
PFHpA	5.0	86.0 ± 4.1	1.1
PFHxS	5.0	100.6 ± 2.0	0.6

Table 4 continued..

PFAS*	Spike concentration (ng mL^{-1})	Recovery ± RSD	MDL (ng g^{-1})
PFOA	5.0	98.0 ± 1.6	0.5
PFHpS	5.0	102.9 ± 2.9	0.9
6:2FTS	5.0	80.6 ± 7.3	1.9
9Cl-PF3ONS	5.0	77.1 ± 8.3	2.0
PFNA	5.0	96.3 ± 4.6	1.4
PFOS	5.0	104.0 ± 1.5	0.5
PFNS	5.0	96.9 ± 4.3	1.3
8:2FTS	5.0	88.3 ± 6.8	1.9
PFDA	5.0	105.7 ± 5.8	1.9
NMeFOSAA	5.0	78.3 ± 3.2	0.8
PFOSA	5.0	98.3 ± 5.7	1.8
PFDS	5.0	98.0 ± 3.3	1.0
NEtFOSAA	5.0	76.6 ± 5.2	1.2
11CL-PF3OUdS	5.0	87.4 ± 5.8	1.6
PFUnA	5.0	102.0 ± 1.5	0.5
PFDoA	5.0	106.3 ± 3.1	1.0
PFTrDA	5.0	93.4 ± 2.7	0.8
PFTA	5.0	100.0 ± 3.5	1.1

*Mass used for all samples is 0.5 g.

25 PFAS in aquatic larval insects that ranged between 0.03 and 0.37 ng g^{-1}; however, these samples contained various insect species and mean internal surrogate recoveries were as low as 40 ± 25 (% ± SD) for some analytes.

The method was applied to analyze 15 composite samples of dragonfly nymphs (n = 1-2 individuals per composite). PFOS was the most frequently detected compound and was found in 100% of nymph samples, followed by PFNA (33.3%), PFOA (33.3%), PFUnA (33.3%), PFHxS (20%), PFDA (13.3%), PFHxA (6.7%), 6:2FTS (6.7%). However, one of the PFHxS detections was below the MDL (0.51 ng g^{-1}). PFOS had the highest detected concentrations ranging from 2.9-62.8 ng g^{-1}, followed by PFOA (0.68-22.51 ng g^{-1}), PFNA (2.24-20.04 ng g^{-1}), 6:2FTS (19.06 ng g^{-1}), PFDA (3.81-4.94 ng g^{-1}), PFUnA (3.22-4.79 ng g^{-1}), PFHxA (3.15 ng g^{-1}), and PFHxS (0.51-1.99 ng g^{-1}) (Figure 3).

CONCLUSION

The validated method presented offers an efficient means of quantifying 28 PFAS compounds in dragonfly nymphs through a combination of QuEChERS extraction and clean-up, followed by UPLC-MS/MS analysis. Notably, this approach stands out for its rapidity and simplicity in sample preparation, obviating the need for traditional gel permeation or solid phase extraction (SPE) typically employed for complex biological matrices. By streamlining sample preparation and enhancing throughput, this method enhances laboratory productivity while reducing associated costs and solvent waste, aligning with the goals of green analytical chemistry [37].

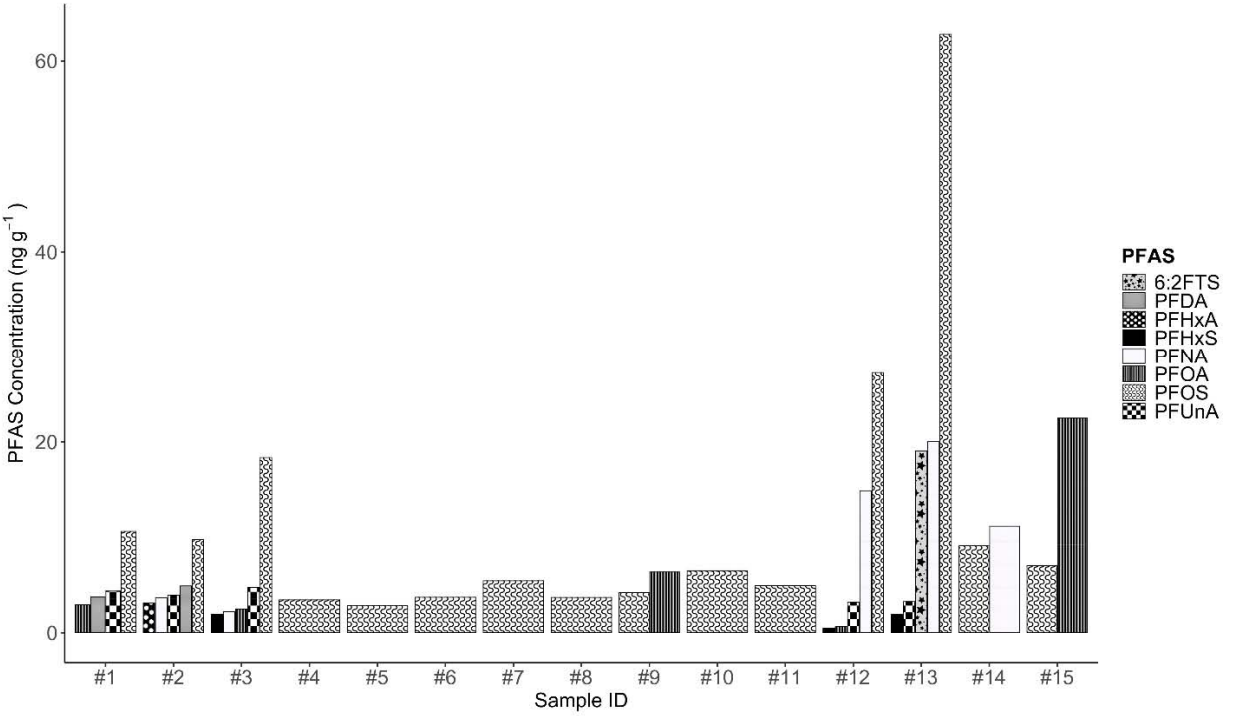

Figure 3. Concentrations (ng g^{-1}) of 28 per- and polyfluoroalkyl substances (PFAS) in aquatic larval odonatan tissue samples (n = 15) using QuEChERS extraction by UPLC-MS/MS. Analytes are defined in the Materials and Methods section.

Application of this method for the analysis of 15 unknown composite dragonfly nymph samples revealed the presence of 8 out of 28 targeted PFAS in at least one sample, indicating PFAS contamination in these organisms. This underscores the potential utility of dragonfly nymphs as valuable biomarkers for PFAS detection in environmental monitoring efforts. Leveraging insects as biomarkers, coupled with advanced analytical techniques, offers insights into the distribution of PFAS in ecosystems, thereby advancing our understanding of their environmental impact.

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AUTHOR CONTRIBUTIONS

Kaitlyn Campbell: Investigation, Methodology, Conceptualization, Writing – original draft.
Jessica Brandt: Funding acquisition, Writing – review & editing.
Austin Pelletier: Methodology, Writing – review & editing.
Christopher Perkins: Funding acquisition, Writing – review & editing.
Anthony Provatas: Supervision, Conceptualization, Methodology, Validation, Writing – review & editing.

CONFLICT OF INTEREST STATEMENT

The authors confirm that this article content has no conflict of interest.

APPENDIX

Table S1. PFAA Mix calibration standards prepared from the *537.1 Method Standard* stock solution [34].

Stock solution used	Initial conc. (ng mL^{-1})	Initial volume (mL)	Final volume (mL)	Dilution factor	Final conc. (ng mL^{-1})	Final name
PFAA_Mix_2000	2000	1	2	0.5	1000	*PFAA_Mix_100*
PFAA_Mix_1000	1000	1	2	0.5	500	*PFAA_Mix_500*
PFAA_Mix_500	500	1	2	0.5	250	*PFAA_Mix_250*
PFAA_Mix_250	250	0.8	2	0.4	100	*PFAA_Mix_100*
PFAA_Mix_100	100	1	2	0.5	50	*PFAA_Mix_50*
PFAA_Mix_50	50	1	2	0.5	25	*PFAA_Mix_25*
PFAA_Mix_25	25	0.8	2	0.4	10	*PFAA_Mix_10*

Table S2. PFAS Mix calibration standards prepared from the Perfluorinated Native Compound Standard (*PFC-24-10X*) stock solution [34].

Stock solution used	Initial conc. (ng mL^{-1})	Initial Volume (mL)	Final Volume (mL)	Dilution factor	Final Conc. (ng mL^{-1})	Final name
ORG_PFASM-1023	20000	1	10	0.1	2000	*ORG_PFASM24_2000*
ORG_PFASM24_2000	2000	5	10	0.5	1000	*ORG_PFASM24_1000*
ORG_PFASM24_1000	1000	5	10	0.5	500	*ORG_PFASM24_500*
ORG_PFASM24_500	500	5	10	0.5	250	*ORG_PFASM24_250*
ORG_PFASM24_250	250	2.5	10	0.4	100	*ORG_PFASM24_100*
ORG_PFASM24_100	100	5	10	0.5	50	*ORG_PFASM24_50*
ORG_PFASM24_50	50	5	10	0.5	25	*ORG_PFASM24_25*
ORG_PFASM24_25	25	4	10	0.4	10	*ORG_PFASM24_10*
ORG_PFASM24_10	10	5	10	0.5	5	*ORG_PFASM24_5*

Table S3. Concentrations (ng mL^{-1}) of MPFHxA, MPFDA, and M3HFPO-DA in the surrogate calibration standards as the dilutions were performed [34].

Stock solution used	Initial conc. (ng mL^{-1})	Initial volume (µL)	Final volume (µL)	Dilution factor	Final conc. (ng mL^{-1})	Final name
EPA-537SS-R1_1	1000	200	400	0.5	500	*EPA-537SS-R1_2*
EPA-537SS-R1_2	500	200	400	0.5	250	*EPA-537SS-R1_3*
EPA-537SS-R1_3	250	160	400	0.4	100	*EPA-537SS-R1_4*

Table S3 continued..

Stock solution used	Initial conc. (ng mL^{-1})	Initial volume (µL)	Final volume (µL)	Dilution factor	Final conc. (ng mL^{-1})	Final name
EPA-537SS-R1_4	100	200	400	0.5	50	*EPA-537SS-R1_5*
EPA-537SS-R1_5	50	200	400	0.5	25	*EPA-537SS-R1_6*
EPA-537SS-R1_6	25	160	400	0.4	10	*EPA-537SS-R1_7*

Table S4. Concentration (ng mL^{-1}) of d5-N-EtFOSAA in the surrogate calibration standards as the dilutions were performed [34].

Stock solution used	Initial conc. (ng mL^{-1})	Initial volume (µL)	Final volume (µL)	Dilution factor	Final conc. (ng mL^{-1})	Final name
EPA-537SS-R1_1	4000	200	400	0.5	2000	*EPA-537SS-R1_2*
EPA-537SS-R1_2	2000	200	400	0.5	1000	*EPA-537SS-R1_3*
EPA-537SS-R1_3	1000	160	400	0.4	400	*EPA-537SS-R1_4*
EPA-537SS-R1_4	400	200	400	0.5	200	*EPA-537SS-R1_5*
EPA-537SS-R1_5	200	200	400	0.5	100	*EPA-537SS-R1_6*
EPA-537SS-R1_6	100	160	400	0.4	40	*EPA-537SS-R1_7*

Table S5. List of per- and polyfluoroalkyl substances (PFAS) analyzed, chemical formulas, and class [34].

Acronym	Compound	Chemical formula	Class
PFBA	Perfluorobutanoic acid	$C_3F_7CO_2H$	PFCA
PFPeA	Perfluoropentanoic acid	$C_5HF_9O_2$	PFCA
PFHxA	Perfluorohexanoic acid	$C_6HF_{11}O_2$	PFCA
PFHpA	Perfluoroheptanoic acid	$C_7HF_{13}O_2$	PFCA
PFOA	Perfluorooctanoic acid	$C_8HF_{15}O_2$	PFCA
PFNA	Perfluorononanoic acid	$C_9HF_{17}O_2$	PFCA
PFDA	Perfluorodecanoic acid	$C_{10}HF_{19}O_2$	PFCA
PFUnA	Perfluoroundecanoic acid	$C_{11}HF_{21}O_2$	PFCA
PFDoA	Perfluorododecanoic acid	$C_{12}HF_{23}O_2$	PFCA
PFTrDA	Perfluorotridecanoic acid	$C_{13}HF_{25}O_2$	PFCA
PFTA	Perfluorotetradecanoic acid	$C_{14}HF_{27}O_2$	PFCA
PFBS	Perfluorobutanesulfonic acid	$C_4HF_9O_3S$	PFSA
PFPeS	Perfluoropentane sulfonic acid	$C_5HF_{11}O_3S$	PFSA

Table S5 continued..

Acronym	Compound	Chemical formula	Class
PFHxS	Perfluorohexanesulfonic acid	$C_6HF_{13}O_3S$	PFSA
PFHpS	Perfluoroheptanesulfonic acid	$C_7HF_{15}O_3S$	PFSA
PFOS	Perfluorooctanesulfonic acid	$C_8HF_{17}O_3S$	PFSA
PFNS	Perfluorononanesulfonic acid	$C_9HF_{19}O_3S$	PFSA
PFDS	Perfluorodecane sulfonic acid	$C_{10}F_{21}SO_3H$	PFSA
4:2FTS	1H,1H,2H,2H-perfluorohexanesulfonic acid	$C_6H_5F_9O_3S$	FTS
6:2FTS	1H,1H,2H,2H-perfluorooctanesulfonic acid	$C_8H_5F_{13}O_3S$	FTS
8:2FTS	1H,1H,2H,2H-perfluorodecanesulfonic acid	$C_{10}H_5F_{17}O_3S$	FTS
HFPO-DA	hexafluoropropylene oxide dimer acid	$C_6HF_{11}O_3$	PFECA
ADONA	4,8-dioxa-3H-perfluorononanoic acid	$C_{10}H_{11}N_4NaO_5S$	PFECA
9Cl-PF3ONS	9-chlorohexadecafluoro-3-oxanonane-1-sulfonic acid	$C_8HClF_{16}O_4S$	Cl-PFESA
11Cl-PF3OUdS	11-chloroeicosafluoro-3-oxaundecane-1-sulfonic acid	$C_{10}HClF_{20}O_4S$	Cl-PFESA
NMeFOSAA	N-Methyl perfluorooctane sulfonamidoacetic acid	$C_{11}H_6F_{17}NO_4S$	FOSAA
NEtFOSAA	N-Ethyl perfluorooctane sulfonamidoacetic acid	$C_{12}H_8F_{17}NO_4S$	FOSAA
PFOSA	Perfluorooctane sulfonamide	$C_8H_2F_{17}NO_2S$	FASA

PFSA = perfluoroalkyl sulfonic acid, PFCA = perfluoroalkyl carboxylic acid, FOSAA = perfluoroalkane sulfonamidoacetic acid, FTS = fluorotelomer sulfonate, Cl-PFESA = chlorinated polyfluoroalkyl ether sulfonic acid, FASA = perfluoroalkane sulfonamide, PFECA = polyfluoroalkyl ether carboxylic acid.

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